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Modeling the Inactivation of *Escherichia coli* O157:H7 on Inoculated Alfalfa Seeds During Exposure to Ozonated or Electrolyzed Oxidizing Water

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Abstract. *Alfalfa sprouts contaminated with Escherichia coli O157:H7 and Salmonella have been implicated in a number of foodborne disease outbreaks in recent years. Seeds are attributed to be the main source of contamination for sprouts. Data from studies on the treatment of E. coli O157:H7 inoculated alfalfa seeds with ozonated and electrolyzed oxidizing (EO) water were used to develop models for predicting inactivation of the pathogen. Treatment times of 0 to 16 min were used for ozonated water at initial concentrations of 0 to 21 ppm. For EO water treatments, 0 to 19 amperage (A) data at treatment times of 0 to 32 min were used to develop the models. A modified Monod model for bacterial death kinetics was developed by integrating the rate constant (k) as a Lorentzian function of treatment time (t). Regression constants for the Lorentzian function were determined at various ozone concentrations (ppm) or A. Validation showed that the model was an effective predictor at ozone concentrations below 8 ppm. As a second method, a response surface model (RSM) was utilized for which an RSM regression was performed between observed* $log_{10}N/N_o$ *and ppm (ozone) or A (EO water) and treatment time. A quadratic equation involving linear, quadratic, and interaction terms of the influencing parameters represented the model for ozonated and EO water treatments. The models were validated by back predicting* $log_{10}N/N_o$ *values. Although numerous other factors influence the accuracy of prediction of the models, these models can be useful tools to researchers and regulators for the development of improved seed sanitizing guidelines by facilitating assessment of efficacy of a treatment and enhancing food safety.*

Keywords. *Alfalfa,* E. coli *O157:H7, Inactivation, Modeling, Ozone, EO water.*

An increase in the consumption of raw sprouted seeds, especially those of alfalfa, has been paralleled by an upsurge in the number of foodborne disease outbreaks in recent years (Taormina et al., 1999; Fu et al., 2001; Lee et al., 2002; Suslow et al., 2002). Seeds are attributed to be the main source of contamination of sprouts, although pathogens may also be introduced during sprout production, harvesting, storage, or transportation (NACMCF, 1999; Holliday et al., 2001; Soylemez et al., 2001; Scouten and Beuchat, 2002). Although good manufacturing practices (GMPs) and hazard analysis and critical control point (HACCP) approaches can reduce the risk of contamination, there is a need to find effective methods to further minimize sprout-associated illnesses (Federal Register, 1999). Ozone and electrolyzed oxidizing (EO) water have emerged as potential sanitizers for decontamination of alfalfa seeds and sprouts. Both reduce the probability of development of potentially carcinogenic residues associated with some other treatments such as 20,000 ppm chlorine (Kim et al., 2000; Sharma et al., 2002; Sharma and Demirci, 2003; Stivarius et al., 2002).

Ozone is a strong antimicrobial agent with high reactivity and spontaneously decomposes to a nontoxic product (i.e., oxygen). It has been used with varied success to inactivate microorganisms on foods such as meat, poultry, eggs, fish, fruits, and vegetables (Graham, 1997; Kim et al., 1999a, 1999b; Stivarius et al., 2002). Relatively low concentrations of ozone and short contact times are sufficient to inactivate bacteria, molds, yeasts, parasites, and viruses in aqueous suspensions (Kim et al., 1999b). However, the presence of food contributes organic matter and may limit the accessibility of ozone to surface and subsurface contaminants, such as those in the crevices of seeds and sprouts, thereby varying the inactivation kinetics (Kim et al., 1999a).

Electrolyzed oxidizing water is generated by the electrolysis of NaCl solution in a chamber where the anode and cathode electrodes are separated by a membrane (Park et al., 2002; Venkitanarayanan et al., 1999). The disinfection

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Mention of brand or firm names does not constitute an endorsement by the USDA over others of a similar nature not mentioned.

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capabilities of EO water depend on its oxidation-reduction potential (ORP), low pH, and the presence of hypochlorous acid (HOCl) and hypochlorite ions. These factors are in turn influenced by the current input to the EO water generator.

Despite the research conducted to investigate disinfection potential of ozone and EO water for inactivation of microorganisms on fresh foods, an understanding of the contributions of individual factors limiting microbial growth and providing safe food products is inadequate (Roberts, 1997). Models developed for microbial death kinetics in broths have been found to be inappropriate for food systems, which are far more complex than broths (McMeekin et al., 1997; Seman et al., 2002). Whiting and Buchanan (1994) classified primary models, such as the Monod (first-order kinetic) model, as those describing the initial microbial population, lag time, growth/death rate, and maximum population density. The inactivation of *Listeria monocytogenes* in milk and pork treated with high pressure was analyzed by the first-order kinetic equation in combination with the Arrhenius model for determining a pressure-dependent rate constant (k) (Mussa et al., 1999a, 1999b). Ludikhuyze et al. (1998) predicted inactivation of soybean lipoxygenase under dynamic conditions using the Monod model with a modified rate constant describing the combined effect of pressure and temperature. Stone et al. (2002), in a nuclear magnetic study of hydrolysis of pyruvic acid, used Lorentzian fit for predicting hydrogen ion concentration. Polynomial models based on response surface methods involve simultaneous determination of the interactive effects of various factors (McMeekin et al., 2002). Seman et al. (2002) showed that under the conditions tested, a central composite second-order response surface model (RSM) could be a useful tool to estimate the amounts of sodium diacetate, sodium, chloride, and potassium lactate required to inhibit the growth of *L. monocytogenes* in meat products. RSMs proposed by Pond et al. (2001) were based on linear, quadratic, and interactive effects of pH, a_w, and time. They showed good correlation between observed and predicted reductions in populations of *E. coli* O157:H7 in uncooked sausages.

A predictive model estimating the inactivation kinetics of *E. coli* O157:H7 on alfalfa sprouts, as a function of concentration of the bactericidal agent in treatment solution, would be beneficial to sprout producers in evaluating the efficacy of sanitizers. The objective of this study was to integrate food microbiology, engineering, and statistics, and develop prediction models (modified Monod model and RSM) on inactivation of *E. coli* O157:H7 on alfalfa seeds with ozonated and EO water.

Materials and Methods

Preparation of *E. coli* O157:H7 Inoculum

Five strains of enterohemorrhagic *E. coli* O157:H7 resistant to nalidixic acid were obtained from the Center for Food Safety, University of Georgia. The strains were: 932 (human isolate), 994 (salami isolate), E0018 (calf fecal isolate), H1730 (human isolate from an outbreak associated with lettuce), and F4546 (human isolate from an outbreak associated with alfalfa sprouts). Cells were grown in tryptic soy broth (Difco, Detroit, Mich.) supplemented with 50 μg/mL nalidixic acid and 0.1% dextrose (TSBN) at 37°C for 18 h. The use of nalidixic acid minimized growth of microorganisms other than *E. coli* O157:H7 in enumeration media used to determine populations of the pathogens on inoculated seeds. A mixture of the five *E. coli* O157:H7 strains was prepared by combining 100 mL of each 18 h culture and centrifuging (Sorvall STH750, Kendro Lab Product, Newtown, Conn.) at 3,300 × *g* for 15 min at 4°C. The supernatant was decanted, and the pellet was resuspended in 300 mL of sterile 0.1% peptone water before centrifuging again at 3,300 × *g* for 15 min at 4°C. The pellet was then resuspended in 1 L of sterile 0.1% peptone water.

Inoculation of Alfalfa Seeds

Non-scarified alfalfa seeds (Lot No. TY12) were obtained from International Specialty Supply (Cookeville, Tenn.). One kilogram of alfalfa seeds was poured into a 2 L beaker containing 1 L of the five-strain suspension of *E. coli* O157:H7 (~10^8 CFU/mL) and soaked for 1 min with continuous gentle, manual agitation using a stirring rod. After the suspension cell solution was decanted, seeds were placed on a sterile perforated tray lined with four layers of cheesecloth and dried in a laminar flow hood at room temperature (21°C ±1°C) for 24 h. Dried seeds with ~10^5 CFU of *E. coli* O157:H7 per gram were sealed in plastic Ziploc bags and stored at 4°C until used within one week.

Preparation of Ozonated Water

Ozone gas (0.34 m^3/h) was generated using a lab-scale ozone generator (model H-50, Hess Machines International, Ephrata, Pa.) equipped with an oxygen concentrator. Two liters of sterile deionized water, at 4°C in a 2 L Erlenmeyer flask fitted with a silicon stopper, inlet, and exit lines, was sparged with ozone through a 10 μm stainless steel sparger for 1 h, to attain 21 ppm aqueous ozone. The undissolved excess ozone gas was passed through 2% potassium iodide solution to prevent it from being released into the environment. The sparging process was performed in a fume hood for safety purposes. In addition, the temperature of water during ozonation was maintained at 4°C, since the solubility of ozone increases with decreased temperature. The ozonated water so prepared was then diluted with sterile deionized water to obtain desired concentrations for treatments. The concentration of ozone in the water was determined by direct measurement of UV absorption at 258 nm, as described in Sharma et al. (2002).

Preparation of Electrolyzed Oxidizing Water

Electrolyzed oxidizing water was produced with an EO water generator (model ROX 20TA, Hoshizaki Electric Co. Ltd., Sakae, Toyoake, Aichi, Japan). A continuous supply of deionized water and 12% sodium chloride solution at room temperature was pumped into the equipment operating at 6, 14, or 19 A. The pH and ORP of the EO water were determined with a pH/ORP meter (model pH 430, Corning, Inc., Corning, N.Y.) using appropriate pH or ORP probes. The pH of acidic EO water used in the study was 2.5, and the ORP was 1,150 mV. Total and free chlorine were determined using the total chlorine test kit and DPD-FEAS (N, N-diethyl-p-phenylenediamine-ferrous ethylenediammonium sulfate) test kit, respectively, according to manufacturer's description (Hach Co., Ames, Iowa).

Table 1. Experimental design for ozonated and EO water treatment of alfalfa seeds.

	Treatment time (min)						
	0	2	4	8	16	32	64
Ozone conc. (ppm)	4, 8, 10, 21	4, 8, 10, 21	4, 8, 10, 21	4, 8, 10, 21	4, 8, 10, 21	4, 8, 10, 21	4, 8, 10, 21
Current levels (A)	6, 14, 19	6, 14, 19	6, 14, 19	6, 14, 19	6, 14, 19	6, 14, 19	6, 14, 19

Treatment of Alfalfa Seeds

Ozonated Water

Twenty-five grams of inoculated alfalfa seeds were soaked in 1 L of ozonated water with continuous agitation using a motorized stirrer rod at a speed setting of 2 (model 4554-00, Cole Parmer, Barrington, Ill.). To investigate the effect of ozone concentration on lethality to *E. coli* O157:H7, water initially containing 4, 8, 10, or 21 ppm ozone was used. These concentrations enabled a study of the effects of a range of ozone concentrations that could be produced with the lab-scale ozone generator. Twenty-one ppm was the highest ozone concentration that could be achieved in a reasonable sparging time of 1 h. Alfalfa seeds were soaked in ozonated water at each concentration of ozone for 2, 4, 8, 16, 32, and 64 min (table 1).

Electrolyzed Oxidizing Water

Twenty-five grams of inoculated alfalfa seeds were treated in 1 L of acidic EO water with continuous agitation using a motorized stirrer rod at a speed setting of 2 (model 4554-00, Cole Parmer, Barrington, Ill.). Three current levels for the EO water generator (6, 14, and 19 A) were used to study the efficacy of various EO water characteristics on viability of *E. coli* O157:H7 on the seeds. Input of 14 A was recommended by the manufacturer for optimum operation, while 6 and 19 A were tested to determine the effects of extreme treatment conditions and for comparison to efficacy data reported by other researchers. It is convenient during commercial application to use amperage rather than chlorine concentration as the parameter of interest. At each amperage level, treatments were administered for 2, 4, 8, 16, 32, and 64 min to determine the effect of treatment time (table 1). Before and after the treatment, the total and free chlorine concentrations in the EO water were determined. The total chlorine concentration before treatment ranged from 12 to 90 ppm, while the free chlorine concentration was 10 to 80 ppm. After treatment, the residual total chlorine was between 3 and 60 ppm, depending on treatment time. There was no free chlorine present in the water.

Microbiological Analysis

To determine the population of *E. coli* O157:H7 on untreated seeds (initial count), 10 g of inoculated seeds were soaked in 40 mL of sterile 0.1% peptone water in a stomacher bag for 2, 4, 8, 16, 32, and 64 min. After pummelling the seeds at 240 rpm for 30 s in a stomacher (model 400, Seward Medical, London, U.K.), the wash solution was serially diluted in sterile 0.1% peptone and surface plated (0.1 mL) in duplicate on tryptic soy agar supplemented with 50 μg/mL nalidixic acid (TSAN). After incubating at 37°C for 24 h, presumptive *E. coli* O157:H7 colonies were enumerated.

Populations of *E. coli* O157:H7 on the ozonated or EO water treated seeds were determined by placing the treated seeds (25 g) in 100 mL of sterile 0.1% peptone water followed by stomaching for 30 s, serially diluting in 0.1% peptone, and surface plating on TSAN. Colonies formed on TSAN from samples of peptone water from untreated and treated seeds were randomly picked and subjected to *E. coli* O157:H7 latex agglutination test (Remel Microbiology Products, Lenexa, Kansas) for confirmation.

Sterile deionized water was used as a control treatment, using the same treatment time and agitation conditions. Each experiment was replicated three times.

Analysis of Data and Modeling

Models were developed using data from experiments designed to determine the efficacy of ozonated and EO water in killing *E. coli* O157:H7 on inoculated seeds. The Monod

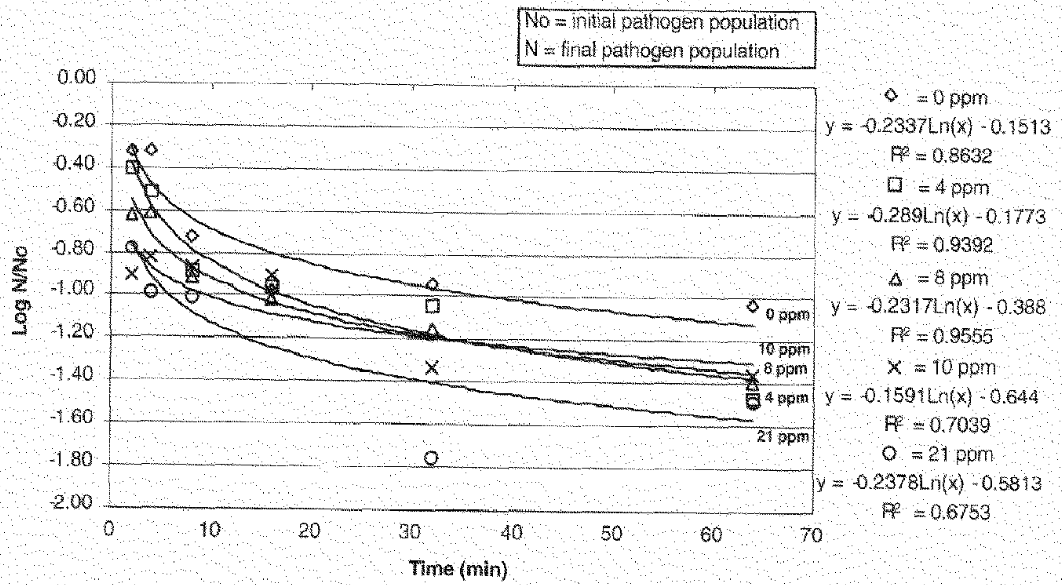

Figure 1. Logarithmic regression of difference in final and initial population of *E. coli* O157:H7 on alfalfa seeds with time at varying ozone concentrations during ozonated water treatment.

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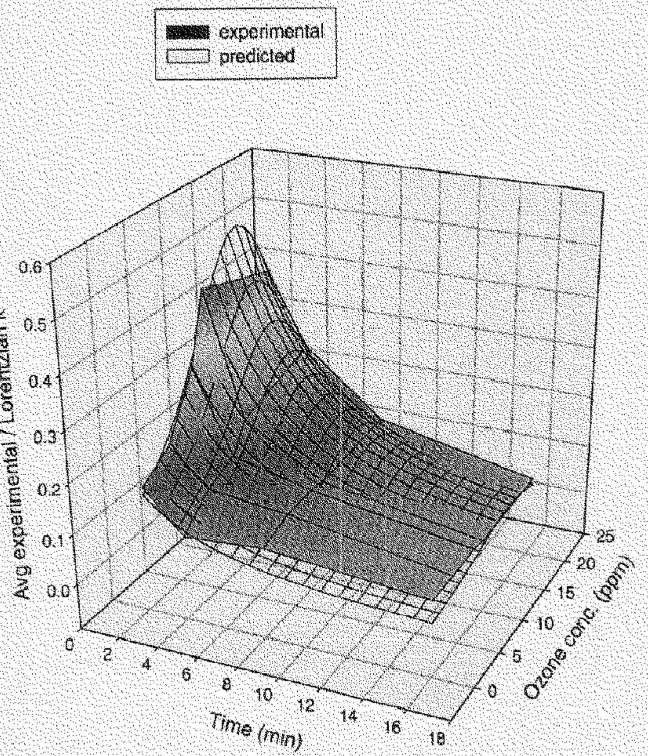

Figure 2. 3D plot of experimental rate constant (k) for alfalfa seeds treated with ozonated water superimposed by Lorentzian regression.

model (eq. 1), in which the rate constant (k) was estimated by Lorentzian regression (Sigma Plot 2001, version 7.0, SPSS, Inc., Chicago, Ill.) (eq. 2), was used to estimate the rate of inactivation of *E. coli* O157:H7 on alfalfa seeds by treatment waters. The Lorentzian function describes a state that undergoes decay exponentially with time (Lehmann, 1998). In other words, it describes a variable whose value follows an exponential relationship with time. Though its application to microbial inactivation studies has not been investigated, regression using the Lorentzian approach, which has a bell-shaped probability distribution with infinite variance, could provide useful predictions of unknown variables. The three parameters in equation 2 (a, b, and x_o) were determined using the experimental rate constant (k) and time (t) for both ozonated and EO water. The values were substituted in the Monod model to obtain predictive equations at each ozone concentration for ozonated water or amperage input for EO water.

$$\frac{dN}{dt} = -kN \tag{1}$$

$$k = \frac{a}{\left[1+\left(\frac{t-x_o}{b}\right)^2\right]} \tag{2}$$

where N is the number of microorganisms at time t, and a, b, and x_o are regression constants. N_o is the initial pathogen population. Analysis showed that 32 and 64 min ozone treatments were not significantly different from 16 min treatments; hence, the data beyond 16 min were not used for modeling. Similarly, for EO water $\log_{10}N/N_o$ values for treatments longer than 32 min were truncated during model development.

As a second method, statistical models were developed for ozonated and EO water treatments using the RSM (Minitab, version 13.30, Minitab, Inc., State College, Pa.). Regression of experimental $\log_{10}N/N_o$ on ozone concentration (ppm) for ozonated water or amperage (A) for EO water with time resulted in equations for prediction of *E. coli* O157:H7 inactivation rates.

Validation of the models was done by back predicting $\log_{10}N/N_o$ values and comparing them with experimental data. A linear regression was performed between the actual and predicted values.

Results and Discussion

The reductions in population of *E. coli* O157:H7 on alfalfa seeds treated with ozonated and EO water at varying conditions were studied. Models involving ozone concentration and time parameters were used to describe the $\log_{10}$ reductions in CFU of *E. coli* O157:H7 per g of seeds. Amperage input for generation of EO water and time were the parameters used for development of EO water treatment models.

Models for Ozonated Water Treatment of Alfalfa Seeds

The difference in final and initial populations of *E. coli* O157:H7 ($\log_{10}N/N_o$) on alfalfa seeds treated with ozonated water at initial ozone concentrations of 0, 4, 8, 10, and 21 ppm for up to 64 min are presented in figure 1. Regression analysis (Microsoft Excel 2000) of values from treatments with each ozone concentration level showed that $\log_{10}N/N_o$ followed a logarithmic trend with time. An analysis of the slopes between N/N_o values at various time intervals showed that there was no significant difference ($P \leq 0.05$) in values for treatments beyond 16 min (Sharma et al., 2002); hence, only values for 2, 4, 8, and 16 min were used for modeling.

Modified Monod Model

In this approach, a modified form of the first-order Monod model developed by using Lorentzian regression was used for predicting the rate of inactivation (death) of *E. coli* O157:H7. The model could be used to predict $\log_{10}N/N_o$ values for specific ozone concentrations up to 8 ppm. Lorentzian regression was performed between the experimental rate constant (k) and treatment time (t) (fig. 2). The robustness of a Lorentzian function eliminates strong influences of extreme outliers by iteratively discarding the ill-fitting data points (NYU, 2002). A good correlation between experimental and predicted k value was observed ($R^2 = 0.971$). The equation for k was substituted into the first-order equation to obtain the following form:

$$\frac{dN}{dt} = -\frac{a}{\left[1+\left(\frac{t-x_o}{b}\right)^2\right]}N$$

The final model has the form as given by equation 3:

$$\log_{10}\frac{N}{N_o} = -a*\left[a\tan((t-x_o)/b)*b + a\tan(x_o/b)*b\right] \quad (3)$$

where a, b, and x_o are regression constants at fixed ozone concentration. Figure 3 gives the values of the regression constants. As the ozone concentration increases beyond the threshold concentration of 4 ppm, a increases. The rate of inactivation is more rapid at higher concentrations of ozone and is associated with greater values of term a. From equation 2, for constant a, t, and x_o, decrease in b would result in decrease in k. However, results indicate that k increases when b decreases. Statistical analysis of experimental data showed that longer treatment time did not result in significantly higher numbers of *E. coli* O157:H7 inactivation; hence, it may be interpreted that b, which is a time-related parameter, does not have a significant effect on k. Additionally, x_o, which is an offset term for t, may neutralize the effect of b.

The model was validated by back predicting $\log_{10}N/N_o$ values resulting from treatments with 0, 4, 8, 10, and 21 ppm ozone and correlating them to the experimental data. A linear regression resulted in R^2 of 0.941, 0.817, and 0.629 for 0, 4, and 8 ppm ozone, respectively (fig. 4). This indicated that at higher ozone concentrations, the model could not sufficiently explain the variability in $\log_{10}N/N_o$ values. Therefore, the model can be used with confidence for ozone concentrations below 8 ppm.

Response Surface Model

RSM analysis with uncoded units (Pond et al., 2001) was used to predict the $\log_{10}N/N_o$ for *E. coli* O157:H7 on alfalfa seeds treated with ozonated water for up to 16 min (fig. 5). A single equation was applied to treatments at five different ozone concentrations and five different times, unlike the previous approach. Variables used to develop the model included time of treatment, t (min), and ozone concentration (ppm). The general equation for the model was of the form:

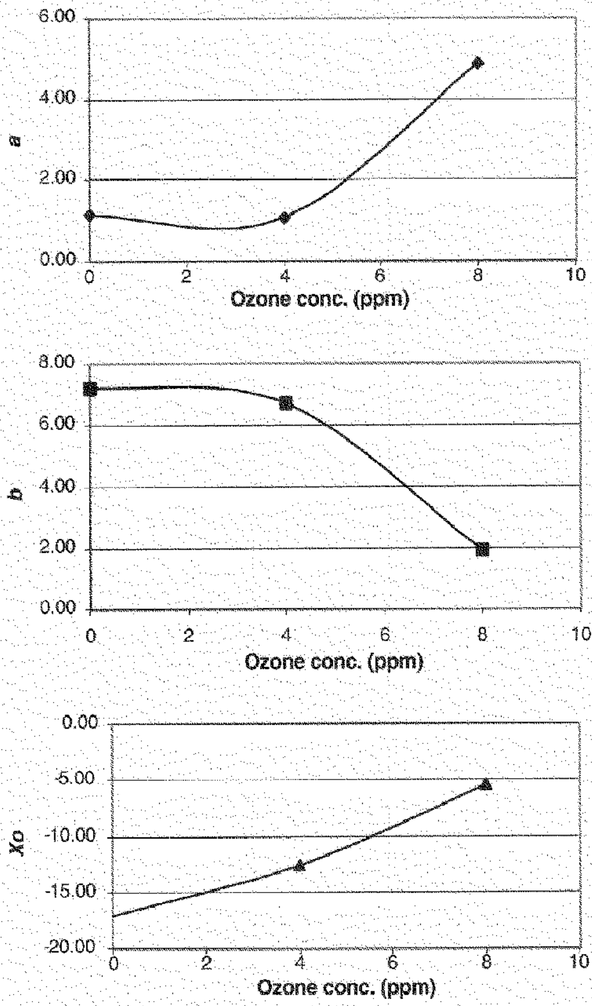

Figure 3. Lorentzian constants (a, b, and x_o) to predict k for treatment of alfalfa seeds with ozonated water at initial concentrations of 0, 4, and 8 ppm.

$$y = \alpha_o + \alpha_1x_1 + \alpha_2x_2 + \alpha_3x_1^2 + \alpha_4x_2^2 + \alpha_5x_1x_2$$

where $y = \log_{10}N/N_o$ for *E. coli* O157:H7 on alfalfa seeds, α_o is the constant term, $\alpha_{1...5}$ are the coefficients, x_1 is the initial concentration of ozone in water, and x_2 is the time of treatment. The data for treatments with 0, 4, 8, 10, and 21 ppm ozone were used to develop the model, and the predictability of the model was validated by back predicting $\log_{10}N/N_o$ values.

$$\log_{10} N/N_o = -0.1051 - 0.0534\text{ppm} - 0.0891t + 0.009\text{ppm}^2 + 0.0023t^2 + 0.0024\text{ppm}\times t \quad (4)$$

The model represented by equation 4 demonstrated a good correlation of 0.848 and slope of 0.99 between the actual and predicted differences in final and initial populations of *E. coli* O157:H7. Analysis of variance of $\log_{10}N/N_o$ showed that all linear, square, and interaction terms had a significant effect ($P \leq 0.05$) on the predictive capability of the model.

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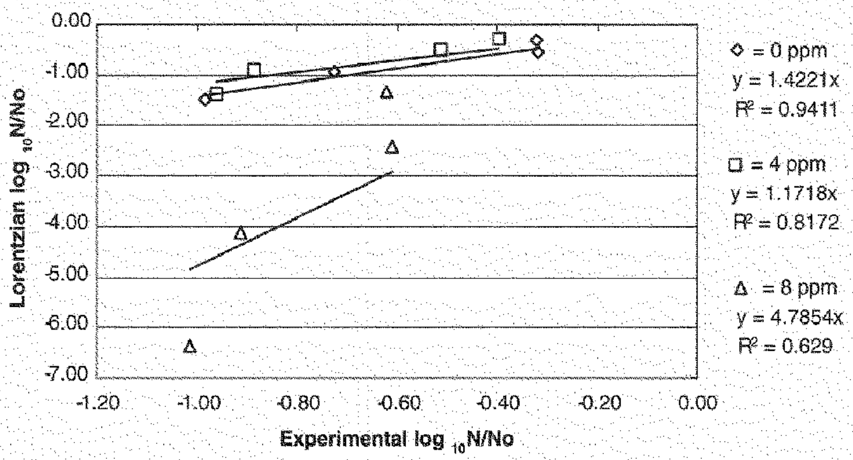

Figure 4. Linear regression of experimental vs. Lorentzian predicted difference in final and initial population of *E. coli* O157:H7 on alfalfa seeds treated with ozonated water at initial concentrations of 0, 4, and 8 ppm.

Models for Electrolyzed Oxidizing Water Treatment of Alfalfa Seeds

Treatment of inoculated alfalfa seeds with EO water generated at various amperages resulted in a logarithmic relation between $\log_{10}N/N_o$ and time of treatment (fig. 6). The slopes between N/N_o values at treatment times up to 64 min revealed that there were no significant effects ($P > 0.05$) of treatments longer than 32 min (Sharma and Demirci, 2003). Therefore, the data were truncated, and $\log_{10}N/N_o$ values for treatment times of 2, 4, 8, 16, and 32 min were used for development of models.

Modified Monod Model

A modified first-order death kinetics model for *E. coli* O157:H7 on alfalfa seeds treated with EO water was developed for 0, 6, 14, and 19 A. As in the case of ozone-treated seeds, the equation for Lorentzian regression between the rate constant (k) and treatment time (t) was integrated into the first-order Monod model (eq. 3). The regression constants determined at each A level are presented in figure 7. Trends illustrate that for EO water treatments (excluding the control), a decreases, while b increases with increase in A. Similar to the modeling outcome of ozonated water treatments, a is the predominant parameter influencing k. The rate constant decreases with decrease in a, while change in b does not significantly affect k.

Validation of the model by back prediction of $\log_{10}N/N_o$ values for *E. coli* O157:H7 populations showed that at higher amperage input levels, the model tended to underpredict. Linear regression between experimental and predicted values gave R^2 values of 0.872, 0.784, 0.584, and 0.9042 for 0, 6, 14, and 19 A, respectively (fig. 8).

Response Surface Model

The RSM described the EO water treatment of inoculated alfalfa seeds at four A levels and five contact times with a single equation. The model (eq. 5) included amperage (A) input for EO water generation and treatment time (t) as the parameters for prediction of difference in final and initial population of *E. coli* O157:H7.

$$\log_{10} N / N_o = -0.116 - 0.0191\text{A} - 0.0759t$$

$$+ 0.0010\text{A}^2 + 0.0016\text{A}t^2 + 0.0001\text{A}t \qquad (5)$$

The response surface diagram in figure 9 shows that the difference in final and initial populations of *E. coli* O157:H7 increases directly with increase in time. Analysis of variance of the terms showed that the linear, quadratic, and interaction terms involving A were not significant ($P > 0.05$). Nevertheless, they were retained since A input was a factor of concern in this study. A coefficient of determination of 0.815 between the experimental $\log_{10}N/N_o$ values, A and time, indicated that 82% of the sample variation could be explained by the model.

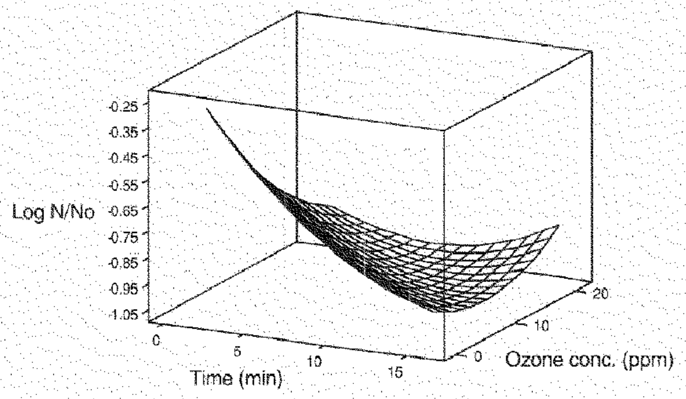

Figure 5. Response surface diagram generated using ozone concentrations of 0, 4, 8, 10, and 21 ppm for the effect of time (min) and concentration on $\log_{10}N/N_o$ for *E. coli* O157:H7 on alfalfa seeds.

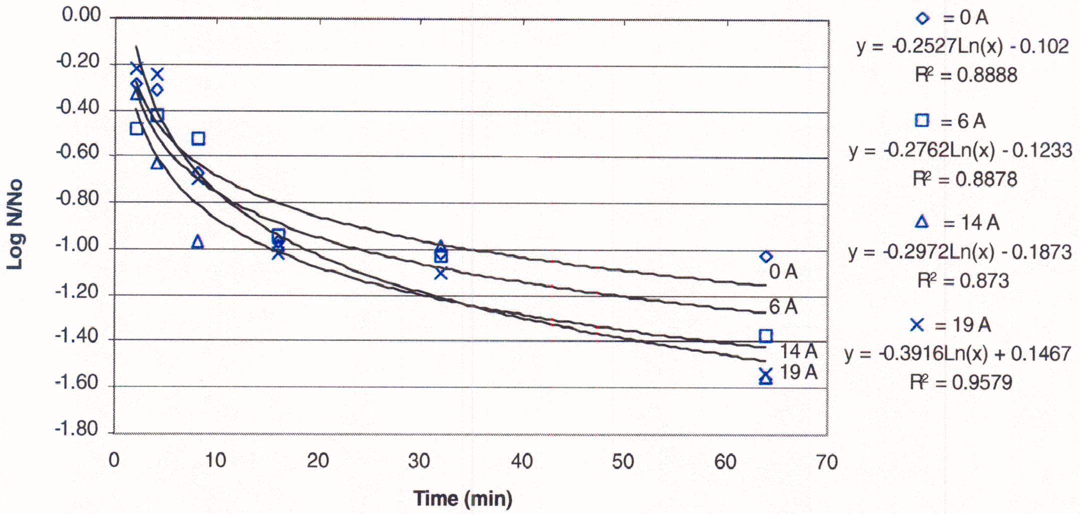

Figure 6. Logarithmic regression of difference between final and initial population of *E. coli* O157:H7 on alfalfa seeds versus time for treatment with EO water generated at various amperages.

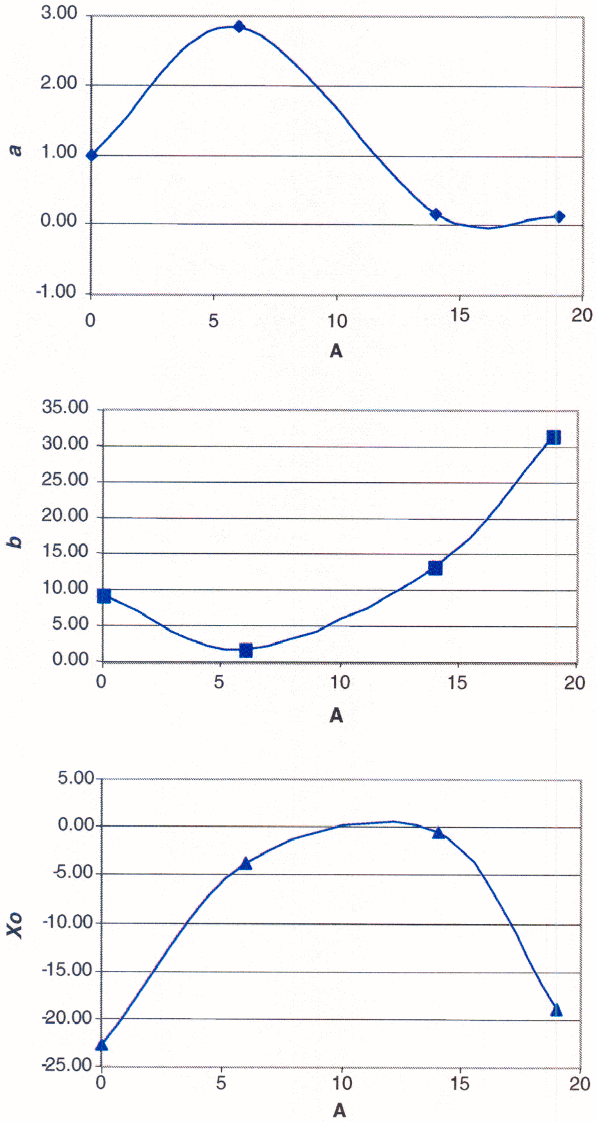

Figure 7. Lorentzian constants (a, b, and x_o) to predict k for treatment of alfalfa seeds with EO water generated at various amperages.

The RSM was validated by linear regression between the values predicted by the model and corresponding experimental data. A good correlation ($R^2 = 0.862$) and slope of 0.95 were obtained between the values, thus showing good predictive capability of the model.

Conclusions

This project aimed at providing models useful to sprout producers for assessing the safety of alfalfa seeds and the type of ozonated or EO water treatment required to inactivate *E. coli* O157:H7 on alfalfa seeds. The models have been validated and are expected to give reliable predictions in the specified ranges. However, it should be noted that the results used for developing these models are specific to the products, techniques, and *E. coli* O157:H7 strains used in this study. Results may vary under different testing conditions (Seman et al., 2002). Extensive investigation of factors such as moisture content of seeds, the ability of the acting agent (ozone/free chlorine) to penetrate the seed surface, and seed size will be required to develop reliable models. Nevertheless, deviations of predicted values relative to observed data do not necessarily imply that the model is faulty; rather, it emphasizes a lack of in-depth knowledge about the biological/food system and its response to antimicrobial treatments (McMeekin et al., 1997). In conclusion, further research is required for development of models that precisely predict the inactivation of pathogens on alfalfa seeds subjected to ozonated and EO water treatments, thereby overcoming the concern of over- or underprediction.

Acknowledgements

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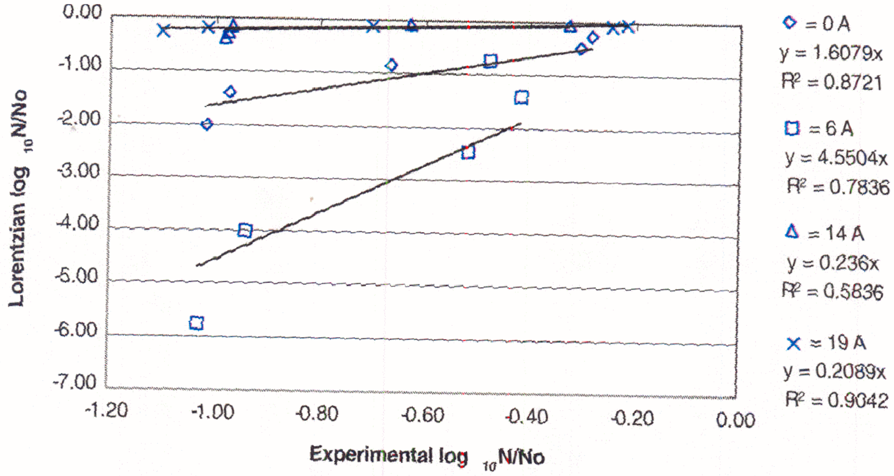

Figure 8. *Linear regression of experimental vs. Lorentzian predicted difference in final and initial population of E. coli O157:H7 on alfalfa seeds treated with EO water generated at various amperages.*

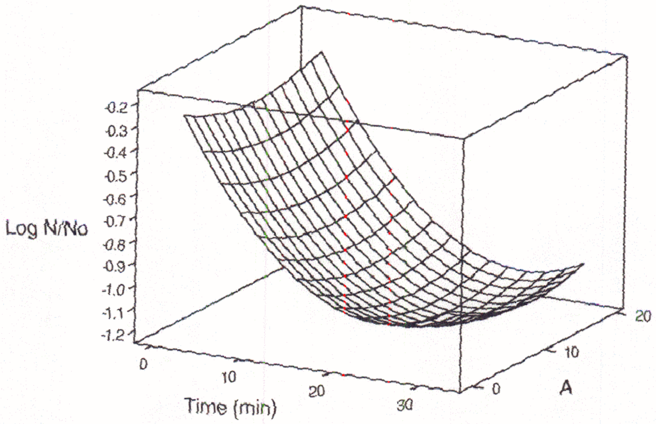

Figure 9. **Response surface diagram generated using various amperages for the effect of time and amperage on $\log_{10}N/N_0$ for *E. coli* O157:H7 on alfalfa seeds.**

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